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An oxidative stress biomarker in acute kidney injury: intra-erythrocyte glutathione

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ABSTRACT

Aims: The aim of the study is to determine intra-erythrocyte glutathione, which is thought to be a more specific and sensitive biomarker apart from the biomarkers traditionally used in the diagnosis of acute kidney injury (AKI), which causes high mortality and morbidity, and is also an oxidative stress marker.

Methods: The study was conducted with 51 volunteers who developed AKI and were diagnosed in the emergency department, and 51 volunteers who applied to the emergency department green triage coded area and did not have AKI. Both groups consisted of individuals between the ages of 18-65, who had a history of diabetes mellitus, who were pregnant, and those who applied to the hospital due to trauma were not included in the study. Demographic characteristics, complete blood count tests at admission, biochemical tests and intra-erythrocyte glutathione and its derivatives were compared in the patient and control groups.

Results: Gender distribution was equal between the groups in the study (p=1,000). Serum urea and serum levels were found to be higher in the patient group compared to the control group. Total glutathione and glutathione disulfide amounts were found to be higher in the patient group, and the native glutathione amount was lower than the control group (p<0.001).

Conclusion: With the results obtained in our study, intra-erythrocyte glutathione and its derivatives in acute kidney injury can be used for diagnostic purposes, since more standardized results can be obtained in AKI than conventional markers. The available data should be supported by more comprehensive studies and considering the limitations of our study.

Keywords: AKI, glutathione, oxidativestress

INTRODUCTION

Acute kidney injury (AKI), defined as a reduction in the kidney's capacity for excretion and filtration over days or weeks, results in the accumulation of waste products that are normally cleared by the kidneys. AKI can manifest asymptomatically, observable only through changes in laboratory parameters, or present as more severe clinical conditions involving alterations in circulating volume, electrolyte disturbances, and/or acid-base imbalance, leading to high morbidity and mortality.[1,2]

In emergency settings, AKI diagnosis spans a wide spectrum, from asymptomatic clinical presentations to mild symptoms such as nausea, vomiting, fatigue, and loss of appetite, and more severe conditions such as uremic encephalopathy, which can cause loss of consciousness.[2]

Serum creatinine, a traditional laboratory marker used in the diagnosis and early treatment of AKI, has been found to lack sufficient sensitivity and specificity due to variations in factors such as age, sex, race, and muscle mass. This has prompted the search for novel biomarkers. In critical illness, the most commonly measured markers of oxidative stress include isoprostanes, hydroxynonenal, lipid peroxides, chlorinated compounds, oxidized glutathione, nitrated and oxidized proteins, and malondialdehyde identified as thiobarbituric acid-reactive substances.[3]

This study aimed to assess intracellular erythrocyte glutathione, an oxidative stress biomarker, as a potentially more specific and sensitive biomarker compared to traditional markers in the diagnosis of AKI, which is associated with high mortality and morbidity.

METHODS

The study was carried out with the permission of Ethical Committe Ankara Bilkent City Hospital Clinical Researches

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Ethics Committee No. 1 (Date: 01.12.2021, Decision No: 2173). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. The study was conducted prospectively as an observational cross-sectional analysis of patients presenting to the Emergency Medicine Clinic at Ankara Bilkent City Hospital.

The study included patients aged 18-65 diagnosed with AKI as the case group and volunteers aged 18-65 presenting to the green triage zone of the emergency department without AKI as the control group. Patients with acute infections, diabetes mellitus, pregnancy, or trauma-related emergency department visits were excluded from both groups.

The diagnosis of AKI was established based on at least a 1.5-fold increase in the patient's serum creatinine level from the baseline, a reduction in glomerular filtration rate (GFR) by at least 25%, and/or a urine output less than 0.5 ml/kg/hour or anuria.

A total of 51 patients meeting the inclusion criteria for AKI and 51 volunteers without AKI were included in the study as the control group.

Statistical Analysis

All analysis was performed using IBM SPSS Statistics for Windows, Version 20.0 (Armonk, NY: IBM Corp). The normality of continuous numerical variables was assessed using the Shapiro-Wilk test. Data with normal distribution were presented as mean±standard deviation and 95% confidence intervals, while data without normal distribution were presented as median (min-max).

The Mann-Whitney U test was used for the comparison of non-normally distributed variables between two independent groups, and the Independent Samples t-test was applied for normally distributed variables. Comparisons of categorical variable ratios were performed using Pearson Chi-Square or Fisher's Exact tests, as appropriate.

Receiver operating characteristics (ROC) analysis was used to determine diagnostic predictive values such as sensitivity and specificity. A p-value <0.05 was considered statistically significant.

RESULTS

Our study included a total of 51 patients diagnosed with AKI and 51 individuals without AKI as the control group. The mean age of the patient group was 59 years, consisting of 25 male and 26 female participants, whereas the control group had a mean age of 43 years, also consisting of 25 male and 26 female participants.

The study groups were compared using biochemical blood tests. The mean blood urea levels were 121 mg/dl in the patient group and 36 mg/dl in the control group, demonstrating a statistically significant difference (p<0.001). For serum creatinine levels, the mean value was 3.1 mg/dl in the patient group and 0.8 mg/dl in the control group, also revealing a statistically significant difference (p<0.001). The glomerular filtration rate (GFR) was compared between the groups, with the mean GFR being 24 ml/min/1.73 m^2 in the patient group and 100 ml/min/1.73 m^2 in the control group, showing a statistically significant difference (p<0.001) (Table 1).

The comparison of native glutathione levels between the groups showed a mean value of 601.639 µmol/L in the patient group and 685.447 µmol/L in the control group, with a statistically significant difference (p<0.001). The total glutathione levels were also significantly different, with a mean value of 1766.060 µmol/L in the patient group and 1413.104 µmol/L in the control group (p<0.001). Furthermore, the mean disulfide level was 44.480 µmol/L in the patient group and 31.961 µmol/L in the control group, indicating a statistically significant difference (p<0.001) (Table 2).

In comparisons of glutathione levels normalized to hemoglobin (Hgb), the mean total glutathione per Hgb was calculated as 133.353 µmol/L in the patient group and 124.376 µmol/L in the control group, with a statistically significant difference (p<0.001). The native glutathione per Hgb was 44.394 µmol/L in the patient group and 60.454 µmol/L in the control group, again showing a statistically significant difference (p<0.001). The mean disulfide per Hgb was 44.480 µmol/L in the patient group and 31.961 µmol/L in the control group, with a statistically significant difference (p<0.001) (Table 3).

Table 1. Biochemical blood analysis of patient and control groups

	Groups										
	Control					Patient					
	Mean	SD	Med	%25	%75	Mean	SD	Med	%25	%75	p-value
Urea	36	14	34	28	44	121	65	103	77	139	<0.001*
Creatinine	0.8	0.2	0.8	0.7	1.0	3.1	1.9	2.4	1.9	3.4	<0.001*
GFR	100	24	99	86	119	24	13	23	15	31	<0.001*

*Mann Whitney-U test, **Independent Samples-t test, SD: Standard deviation, GFR: Glomerular filtration rate

Table 2. Total glutathione, native glutathione, and disulfide analysis in patient and control groups

	Groups										
	Control					Patient					
	Mean	SD	Med	%25	%75	Mean	SD	Med	%25	%75	p-value
Native glutathione	685.447	253.891	674.880	538.017	747.918	601.639	210.257	576.870	456.960	719.460	<0.001*
Total glutathione	1413.104	314.131	1347.005	1198.131	1659.711	1766.060	245.268	1697.250	1599.693	1842.020	<0.001*
Disulfide	363.828	140.299	341.084	260.913	451.096	582.211	169.923	583.934	509.846	670.025	<0.001**

*Mann Whitney-U test, **Independent Samples-t test, SD: Standard deviation

Table 3. Glutathione and disulfide levels per hemoglobin in patient and control groups

	Groups										
	Control					Patient					
	Mean	SD	Med	%25	%75	Mean	SD	Med	%25	%75	p-value
Native Glutathione per Hgb	60.454	22.871	57.403	44.393	72.380	44.394	13.680	42.417	33.315	52.364	<0.001**
Total Glutathione per Hgb	124.376	32.685	119.762	98.512	146.196	133.353	28.417	126.637	111.394	152.194	<0.001*
Disulfide per Hgb	31.961	13.573	27.986	22.079	41.944	44.480	16.238	42.610	34.071	53.821	<0.001*

*Mann Whitney-U test, **Independent samples-t test, SD: Standard deviation, Hgb: Hemoglobin

DISCUSSION

AKI has an incidence rate of 18% among hospitalized patients and 0.25% in the general population. While the mortality rate for uncomplicated AKI is 5-10%, this figure can rise to 40-90% in intensive care settings, highlighting AKI as a significant public health problem.[3,4]

In our study, we prospectively analyzed data from 51 patients diagnosed with AKI or chronic kidney disease exacerbated by AKI who presented to the Emergency Department of Ankara Bilkent City Hospital, along with 51 patients without AKI confirmed by laboratory tests.

The gender distribution in both the patient and control groups was equal. The mean age of the patient group was 51 years, while the control group had a mean age of 43 years. The higher mean age of the patient group may be attributed to the selection of the control group from the green triage zone.

Serum creatinine, traditionally used in the diagnosis and early treatment of AKI, has been found to lack adequate sensitivity and specificity due to variations related to age, gender, race, and muscle mass. Consequently, alternative biomarkers have been explored. Commonly measured oxidative stress markers in critical illness include isoprostanes, hydroxynonenal, lipid peroxides, chlorinated compounds, oxidized glutathione, nitrated and oxidized proteins, and malondialdehyde, a thiobarbituric acid-reactive substance.[3]

Glutathione, the most abundant intracellular antioxidant molecule, is synthesized in all eukaryotic cells, primarily in the liver. It plays a critical role in scavenging free radicals, reducing oxidized products, and protecting biomolecules from the harmful effects of reactive oxygen species.[5-7] Conditions that result in cellular stress disrupt protein stabilization and cause lipid peroxidation, leading to the formation of reactive oxygen species. Due to its high metabolic activity, the kidney is one of the organs most affected by oxidative stress.[8]

An animal study demonstrated that renal glutathione (GSH) levels decreased following ischemic injury. Furthermore, administration of N-acetylcysteine increased GSH levels and alleviated AKI.[9]

In our study, oxidative stress was evaluated by measuring total glutathione, native glutathione, and glutathione disulfide levels. Results indicated that the mean disulfide and total glutathione levels were statistically higher in the patient group compared to the control group, whereas the native glutathione levels were significantly higher in the control group than in the patient group.

A study by Otal et al.[10] in Ankara in 2018, involving 42 patients diagnosed with AKI and treated with hemodialysis in the emergency department, along with 45 controls, reported that native glutathione, disulfide, and total glutathione levels were higher in the control group. However, the ratio of disulfide to total glutathione and native glutathione was found to increase in favor of disulfide in AKI patients. The same study demonstrated that these ratios decreased after hemodialysis compared to pre-dialysis levels.

Similarly, a study conducted by Ayar et al.[11] in Ankara between 2015 and 2017, involving 20 pediatric patients with AKI and 39 healthy controls, showed that the median total glutathione and native glutathione levels were significantly lower in the AKI group compared to the control group.

When comparing the results of our study to the literature, it is evident that our findings support previous research.

CONCLUSION

AKI is a clinical condition diagnosed in emergency departments and associated with high morbidity and mortality. Our findings demonstrate that native glutathione levels decrease while glutathione disulfide levels increase in AKI. These parameters may serve as potential biomarkers for AKI, particularly when serum creatinine levels, influenced by various factors, are insufficient for diagnosis. However, considering the limitations of our study, further research with larger sample sizes is required.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of Ethical Committe Ankara Bilkent City Hospital Clinical Researches Ethics Committee No. 1 (Date: 01.12.2021, Decision No: 2173).

Informed Consent

All patients signed and free and informed consent form.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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